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SOLUBLE NONPROTEIN NITROGEN OF SOIL

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INTRODUCTION

Dilute alkali dissolves a larger proportion of the organic material of soil than any of the other relatively mild reagents. A still larger percentage is extracted from soil previously treated with .1 per cent of hydrochloric acid (HCl), and this latter reagent dissolves but little of the organic material. The term "humates" is fast disappearing from current scientific literature, yet one often reads that the reason the preliminary washing with acid renders the organic matter more soluble in the alkali is that the calcium of the calcium humates is dissolved out, making the free humic acids soluble in the alkali. To say that the proteins are rendered more soluble by the removal of the calcium and the heavy metals would explain the solubility just as well and would be more correct scientifically.

As pointed out by Lipman (4, p. 251), much of the organic nitrogen of the soil must be protein in nature. The chief sources of the nitrogen are crop residues, manures, and bacterial cells, and in these much of the nitrogen is in the form of protein. Investigations carried out in this laboratory (5) have shown that soils contain a large quantity of the so-called humin compounds. These have a great tendency to be adsorbed by such compounds as magnesium oxid and calcium hydroxid, and therefore removal of calcium from the soil by acid would tend to make these more soluble.

Upon the acidification of the alkali extract a precipitate is obtained which has been called humic acid. This term also is no longer taken seriously. It would seem that the rational explanation of this precipitate would be simply that it was made up of proteins thrown down, as salts of the precipitant, as salts of organic acids, such as nucleic acid (7), or resinous acids (6), both of the latter substances having been isolated from the acid precipitate. It would also contain, no doubt, some free organic acids.

In analyses of the solution obtained by the prolonged boiling of soils with strong acids and of the hydrolyzed humic acids by the Van Slyke method (8), it was found that the results for the humic acids did not differ markedly from the results for the organic matter of the soils as a whole from which they were derived. Since that time it has occurred to

the writers that this would hold for the material precipitated by acid from the alkali extract, but perhaps this would not be true of the organic nitrogen compounds remaining in solution. It has been pointed out by Shorey (7) that many organic compounds have been isolated from the alkali extract of soil, which, though relatively quite soluble in water, can not be detected in or isolated from the water extract of soils. Therefore it has seemed that information might be obtained relative to the degree of decomposition of the organic matter in the soil by determining the proportion of nitrogenous compounds left in solution after the precipitation of the proteins by a suitable reagent was completed. It was with these problems in mind that the preliminary investigation was carried out.

EXPERIMENTAL METHOD

The general procedure followed was to determine the nitrogen in the alkali extract of soil with and without added material and the determination of nitrogen in the filtrate from the precipitate of the proteins in the alkali extract of soil with and without added material. The recent critical examination of a few protein precipitants by Greenwald (3) led us to use trichloracetic acid as our precipitant.

The detailed procedure was as follows: Soil, ground to pass a sieve of 100 meshes to the inch, was extracted with 1 per cent of hydrochloric acid until no calcium was found in the wash water. After air drying, 100 gm. were placed in an 800 c. c. bottle and 500 c. c. of a 1.5 per cent solution of sodium hydroxid added. After shaking the mixture for 2 hours it was centrifuged for 5 minutes in a bowl centrifuge having a speed of 18,000 revolutions per minute. Two 25 c. c. portions of the clear but deeply colored extract were analyzed for total nitrogen by the Gunning-Arnold method. Two 25 c. c. portions were neutralized with sulphuric acid, sufficient trichloracetic acid in solution added to give a 2.5 per cent solution of the acid and a total volume of exactly 30 c. c. After centrifuging, 10 c. c. portions were taken from each tube and analyzed for nitrogen by the Bock and Benedict (1) modification of the Folin and Denis method (2). This was called the soluble nonprotein nitrogen.

The same procedure was used where material was added to the soil. In the case of guanin, hypoxanthin, and glucosamin,[1] weighed portions of the compounds were added to the soil, which was then shaken with the alkali. The hydrolyzed casein was prepared as directed by Greenwald (3), which consisted, in brief, of boiling the casein for 40 hours with hydrochloric acid, the removal of the acid under diminished pressure, neutralization with sodium hydroxid (NaOH), and filtration. After mixing the filtrate with animal charcoal it was again filtered and final filtration carried out after crystallization of the tyrosin. For all the remaining materials solutions were prepared, sometimes with the aid of a little *N/10* acid or *N/10* alkali. Suitable amounts of the solutions were

[1] We wish to express our thanks to Prof. P. A. Kober for the samples of the hypoxanthin and guanin and to Dr. A. W. Dox for the sample of glucosamin.

added to the soils and then sufficient alkali added to make 500 c. c. of a 1.5 per cent solution. In all cases, with the above-noted exceptions, the purest commercially available compounds were used, but analyses for nitrogen were run on the solid material when it was used, and when solutions were employed aliquots were analyzed. These determinations were also made by the micro method. It should be mentioned here that 6 minutes was found to be quite an inadequate digestion period for some of the compounds. It is believed that in some cases when apparently more than 100 per cent of the added substance was extracted from soil, faulty analysis of the substance was the cause. Insufficiency of material precluded repeating tests with many of the materials.

The soil used for all these tests was a silt loam containing 0.30 per cent of nitrogen. Samples A and B, as shown in Table I, differ only in that they were not taken from the field at the same time.

TABLE I.—*Analyses of 5-gm. portions of soil for alkali-soluble and soluble nonprotein nitrogen*

Soil sample.	Substance added.	Nitrogen added.	Nitrogen in the alkali extract.	Nitrogen of the added substance recovered in the alkali extract.		Soluble nonprotein nitrogen.	Soluble nonprotein nitrogen recovered.	
		Mgm.	*Mgm.*	*Mgm.*	*Per ct.*	*Mgm.*	*Mgm.*	*Per ct.*
A	Nothing	.....	5.93	.....	.....	1.29	.....	.....
	Hydrolyzed casein	2.09	7.99	2.06	98.4	3.33	2.04	97.5
	Amino benzoic acid	2.14	8.08	2.15	100.4	3.41	2.12	99.0
	Glutamic acid	2.27	8.26	2.33	102.6	3.48	2.19	96.5
	Hippuric acid	2.42	8.40	2.47	102.0	3.73	2.44	100.8
	Glutamic acid imid	2.36	8.36	2.43	103.0	3.59	2.30	97.5
	Succinimid	2.53	8.45	2.52	99.5	3.86	2.57	101.5
	Guanidin sulphate	1.97	6.725	1.975	40.4	1.84	0.55	28.0
	Urea	2.48	8.41	2.48	100.0	3.66	2.37	95.6
	Uric acid	2.42	8.34	2.41	99.6	3.78	2.49	103.0
	Caffein	2.32	6.93	1.00	43.1	2.21	.92	39.7
	Theobromin	1.96	7.90	1.97	100.5	3.27	1.98	101.0
	Guanin	2.49	8.45	2.52	101.2	2.325	1.035	41.6
	Hypoxanthin	2.52	8.34	2.42	96.0	3.42	2.13	84.6
	Skatol	1.26	7.19	1.26	100.0	1.80	.51	41.2
	Nucleic acid	2.00	7.92	1.99	99.5	1.67	.38	19.0
	Cadaverin	2.00	7.92	1.99	99.5	2.45	1.16	58.0
	Amygdalin	2.48	8.41	2.48	100.0	3.42	2.13	85.7
	Peptone (Witte)	2.98	8.76	2.83	94.9	2.12	.83	33.5
	Casein	2.42	7.42	1.49	61.6	1.29	0	0
	Edestin	2.43	6.58	.65	26.8	1.29	0	0
	Egg albumin	2.01	7.56	1.63	81.3	1.29	0	0
	Glucosamin	1.98	8.08	2.15	108.5	3.36	2.07	104.5
B	Nothing	.....	6.51	.....	.....	1.25	.....	.....
	Asparagin	2.11	8.62	2.11	100.0	3.24	1.99	94.2
	Acetanilid	2.14	8.66	2.15	100.5	3.24	1.99	93.0
	Benzamid	2.17	8.69	2.18	100.5	3.27	2.02	93.2
	Creatinin	2.07	8.06	1.55	74.8	2.71	1.40	70.5

It is not thought that all the compounds used are actually present in soil. The substances were chosen rather to represent classes of compounds which conceivably might be in soils. Guanin, hypoxanthin, nucleic acid, peptone, and creatinin have been isolated from soil. It is

realized that the list is very incomplete. As opportunity to make or to purchase more compounds presents itself, the investigation will be considerably extended. From the data presented, it is observed that quite varying proportions of the pure proteins, which in reality are soluble in dilute alkali, are extracted. This seems to be a confirmation of the contention that the alkali extract as a whole does not represent a definite class of nitrogenous compounds. Of the simpler compounds, it is seen that the more acid and more closely neutral compounds are completely extracted and remain in the soluble nonprotein portion. An exception to this is found in the case of nucleic acid. This is to be expected from its tendency to combine with protein compounds to give insoluble nucleins. The action of the purin compounds is interesting. In general the more basic the compound the less the quantity recovered.

CONCLUSIONS

(1) If the results with the pure proteins be considered, it is probable that the alkali extract as a whole contains no definite group of compounds.

(2) From the results obtained by the precipitation of the alkali extract with trichloracetic acid it would seem that the soluble nonprotein fraction may contain most of the simpler nitrogenous compounds, and therefore its determination would give an index of the degree of decomposition of the organic matter in the soil.

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OVIPOSITION OF MEGASTIGMUS SPERMOTROPHUS IN THE SEED OF DOUGLAS FIR

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The larva of a seed chalcidid, *Megastigmus spermotrophus* Wachtl, has been very commonly recorded from the seeds of Douglas fir (*Pseudotsuga taxifolia*), but most of these records apply only to mature seed. The method by which the larvæ of this insect get into the seeds has not been previously described. The oviposition of the female, the period of the growth at which the seeds are infested, and the subsequent development of the larvæ are matters on which we have no published data.

The following is an account of the oviposition of this species observed at the Forest Insect Seed Station of the Bureau of Entomology at Ashland, Oreg., during the season of 1915.

During the season of 1914 a heavy emergence of adults of *Megastigmus spermotrophus* from Douglas fir seed of the 1913 crop occurred in the vicinity of Ashland. From stored seed kept in a rearing box the male adults began to emerge on April 12, and the females on April 16; 2,897 adults emerged from 6¾ ounces of seed, the period of maximum emergence occurring between April 23 and May 11. A number of these adults were liberated in a small cage kept in the laboratory. It was found that the adults would not live any length of time unless fed. Pieces of blotting paper saturated with sugar solution were hung in the cage and on this the adults were frequently seen feeding. Young Douglas fir cones were kept in the cage with the adults for a period of about three weeks; and although copulation was frequently observed, no attempts were noted on the part of the females to oviposit in the cones.

During the season of 1915 another effort to secure a record of oviposition in a rearing cage met with far better success. Various lots of infested Douglas fir seed were kept at the station, and the emergence of the adults from this seed was quite similar to that observed in 1914. The maximum period of emergence in the laboratory occurred between April 20 and May 2. From cones which were kept caged over winter under outdoor conditions at the same elevation, the maximum emergence occurred between May 1 and 16. At elevations of 3,000 to 4,000 feet the emergence occurred during the latter part of May, and above 4,000 feet much of the emergence occurred in June.

Many adult chalcidids were liberated at different dates between April 18 and May 20 in a cage considerably larger than that used in 1914 (Pl. V,

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Vol. VI, No. 2
Apr. 10, 1916
K—28

fig. 1). This was kept outdoors in a partially shaded position. The adults were fed as before with sugar solution.

A Douglas fir branch bearing cones about 3 weeks old was placed in this cage on April 18. The young cones were then about 1½ inches long, the scales were still soft, and the seeds had the milky interior and unhardened coat. The base of the branch was kept in a jar full of moist earth. Fresh branches were placed in the cage at intervals until May 15, when the cones were estimated to be about half mature.

Mating was observed in this cage and in the emergence vials of the rearing boxes during the entire period. The first oviposition of a female on a cone was observed on April 20 at about 3.30 p. m. A female was observed crawling about over the bracts and feeling the scales with her antennæ. This lasted for several minutes; then the female paused on one of the exposed scales with her head pointed toward the base of the cone. After resting quietly for a moment the abdomen was lifted and at the same time the posterior end was doubled under so that the sheath of the ovipositor was brought forward between the legs until the tip rested on the surface of the cone scale at a point directly under the insect's head. The point chosen for the insertion of the ovipositor was close to the outer edge of the scale on which the female rested. The sheath of the ovipositor was then withdrawn and assumed its normal position back of the abdomen, while the ovipositor was slowly forced down into the cone. The abdomen was gradually lowered as the ovipositor was thrust into the cone until finally the entire body rested close to and in a line parallel with the surface of the cone scale (Pl. VI, fig. 3). In this position the female rested for about a minute and then withdrew the ovipositor. This was accomplished by raising the body and doubling the abdomen until it assumed a position similar to that in which the oviposition was started (Pl. VII, fig. 1). This allowed the ovipositor to be withdrawn and returned to its sheath.

The oviposition of two females was recorded on April 22 and that of the same number on April 23. Between this date and April 26 no oviposition and very little activity on the part of the seed chalcidids were observed. On the morning of April 26 a female was observed ovipositing, and this operation was recorded four times during the day. On April 27 about the same activity occurred. April 28 was a warm, sunny day and great activity on the part of the females occurred. The cage at this date contained 10 cones and about 50 females. At almost any hour during the day from one to three females could be seen either ovipositing on the cones or preparing to do so. From April 29 to May 2 cool rainy weather prevailed, and almost no activity on the part of the chalcidids occurred in the cage. May 3, 4, and 5 were warm, sunny days, and the oviposition could be witnessed at any time during the day. Oviposition in the 10 cones in the cage on these dates was in progress continuously during the day, at which time the best observations of the act were obtained.

A spell of rainy weather persisted from May 8 to 25, and no further records were secured. The subsidence of emergence after the latter date made it impossible to obtain adults for liberating in the rearing cages, and efforts to secure further records were not attempted.

Difficulty was encountered in securing photographs, as females will not oviposit if even slightly disturbed. If a cone was jarred in any way while a female was in the act, the ovipositor would be withdrawn as rapidly as possible. Even though the ovipositor was inserted deep in the cone the female would struggle to disengage it and fly away. However, by raising the glass on the front of the cage it was possible to focus a camera directly on the cones, and several pictures were obtained in this way. For the purpose of further study and dissection, a number of females were captured and killed with the ovipositor thrust into the cones. This was best accomplished by quickly immersing the cone on which the female rested in a graduate filled with chloroform. This killed the female so quickly that her efforts to withdraw the ovipositor were seldom successful. Several of the females which were killed in this position were photographed (Pl. VI, fig. 1, 2).

The time required for oviposition varies from two to five minutes. The same female was observed to oviposit five times on the same cone, and it is probable that the operation is repeated many times before the egg-laying capacity is exhausted. The point selected for the insertion of the ovipositor was always on the surface of a scale, never on a bract, and may be either on the margin or near the center of the scale. The female always assumed a position with head pointed toward the base of the cone. As Douglas fir cones were pendent at the time of oviposition, this allowed the female to stand with her head pointed upward (Pl. V, fig. 2, 3).

In cones which were dissected with the ovipositor of the female inserted it was found that the ovipositor reached the seed in a few cases only. Apparently where successful the ovipositor passes through the scale nearest the surface and underlying bracts until it reaches the second or third scale from the surface. It then follows down through the center of the last scale nearly to its base and then turns forward into the seed just ahead of it (Pl. VII, fig. 2, 3). The fact that the ovipositor was seldom found in the seed in the cones dissected is doubtless due to the fact that the female partly withdrew her ovipositor in the death struggle.

It would seem that successful oviposition occurs only when the egg is deposited in the seed, as the larvæ have never been found to work their way through the tissues of the cone, and their development is confined entirely to the interior of one seed.

Numerous cases were found in which the ovipositor did not penetrate even as far as the base of the scale. This occurred most frequently where the cones were of such an age that the scales had hardened. In these cases the tough tissues of the scales seem to bend the ovipositor out of

its course, and in a number of the dissections the ovipositor was bent and twisted around to a course directly opposite to that intended. This condition was not encountered where the cones were still young and soft. In fact, after the cones become hardened it is difficult to realize how they can become infested at all by the chalcidids.

Actual oviposition in the field by the seed chalcidids was observed only once—on May 28 by Entomological Ranger J. E. Patterson. While collecting cones he noted a female on one cone with her ovipositor inserted. The insect withdrew the ovipositor and left the cone very soon after it was noticed.

PLATE V

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1.—Type of cage in which the oviposition of *Megastigmus spermotrophus* was observed. This cage was kept under outdoor conditions. A branch bearing young cones of Douglas fir was set in a jar of moist soil and kept in the cage with the females.

Fig. 2, 3.—Female resting on cone with ovipositor inserted. Photographed from life. On left, original; on right, enlargement of same to show exact attitude of the female.

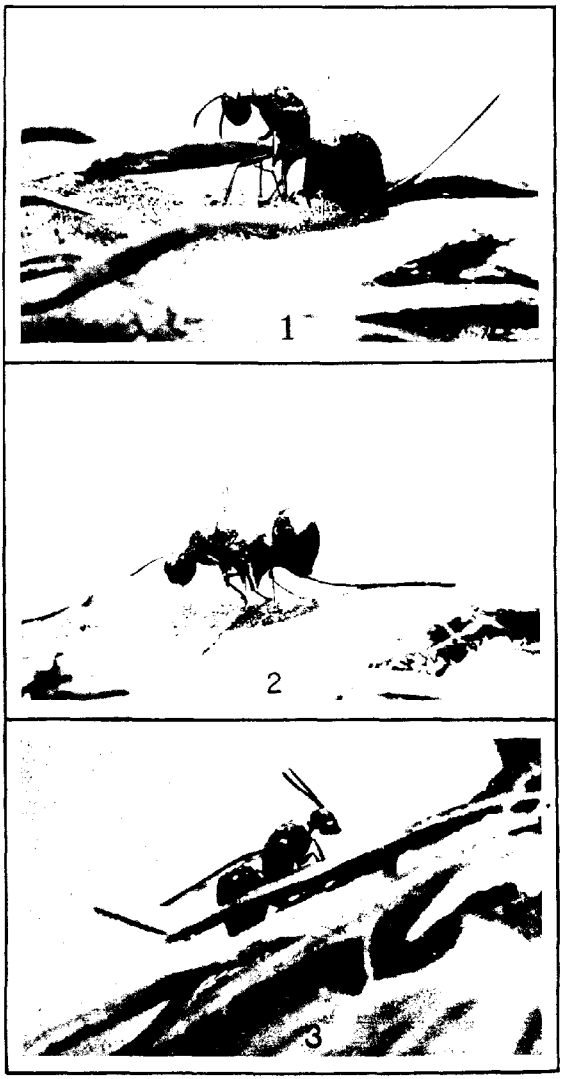
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PLATE VI

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1, 2.—Two positions of female on surface of cone with ovipositor inserted. Photographed from dead females which had been killed in this position. Enlarged.

Fig. 3.—Female resting on cone with ovipositor inserted. Photographed from life. Enlarged.

PLATE VII

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1.—Female in act of withdrawing ovipositor from cone. Photographed from life. Enlarged.

Fig. 2.—Section through a Douglas fir cone on which a female has been killed while in the act of ovipositing.

Fig. 3.—A portion of same cone and dead female with ovipositor inserted. Slightly retouched to show course followed by ovipositor in reaching the seed.

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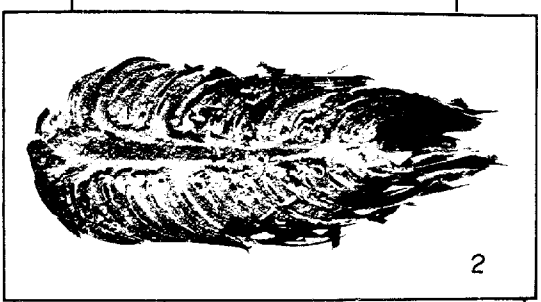

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CITRUS CANKER[1]

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INTRODUCTION

The ravages of certain insect pests and plant maladies have, in a considerable number of instances, been so severe as to cause intense alarm. It has been feared in the case of several crops that their culture was no longer possible in certain sections because effective means of preventing the losses resulting from such ravages were not then known. Within the last two years it has been realized that a new disease known as Citrus canker has been introduced into the Citrus-growing sections of the Gulf Coast States. This disease, beyond all doubt, is the most destructive malady affecting species of Citrus, and when it was realized that its control and eradication were so difficult, alarm concerning the future production of Citrus fruits became almost an hysteria. Those who have never seen Citrus canker under field conditions regard the reports of the highly infectious nature of this disease, of its destructiveness, and of the difficulties experienced in its eradication as the results of an overwrought imagination. The severity of Citrus canker has not been exaggerated, however, and growers should lose no time in preventing its further dissemination and in effecting its eradication.

HOSTS OF THE ORGANISM

Citrus canker has been found to affect many of the varieties and species of Citrus, and in all probability none of the species of this genus are entirely immune. It is perhaps productive of more serious injury to the varieties of grapefruit, or pomelo (*Citrus decumana*), than to any other of the Citrus fruits. Seedling grapefruits appear to be more susceptible to canker than the budded varieties. Some regard the injury to the hardy or trifoliate orange (*Citrus trifoliata*), which is extensively used as the stock upon which to bud other species of Citrus, as equally severe. Certain of the varieties of round oranges (*Citrus aurantium*) are known to be very susceptible to Citrus canker and under favorable conditions suffer as severe injury as grapefruits. The disease occurs also on varieties of the sweet orange. Oranges of the mandarin group (*Citrus nobilis*),

[1] Published with the permission of the Director of the Alabama Experiment Station.

[2] The writer is greatly indebted to his colleague, Dr. J. S. Caldwell, for suggestions and material aid during the progress of this investigation and for assistance in the preparation of the manuscript. Much of the chemical portion of the investigation would have been impossible but for the skillful and arduous assistance of Messrs. A. C. Foster and C. W. Culpepper, formerly laboratory aids in the Department of Botany of the Alabama Polytechnic Institute. To each of these gentlemen grateful appreciation for the several services is hereby acknowledged.

including mandarins, tangerines, and Satsumas, have also been found to be diseased. The disease has been observed, too, on several varieties of lemons (*Citrus medica*) and limes (*Citrus limetta*). Thus far Citrus canker in Alabama has not been found to attack kumquats, the four species of which Swingle (17)[1] regards as belonging to the genus Fortunella. It has been observed, however, on the leaves and twigs of the kumquat in Louisiana. Swingle (18) reports its occurrence on this host in Japan.

HISTORY OF THE DISEASE

Citrus canker is not of American origin, but beyond doubt was introduced into the Gulf States from Japan. This statement is supported by the fact that it is known to occur in Japan and the Philippine Islands (18), and, so far as can be learned, it appeared in the United States several years ago simultaneously with the importation of Satsuma and trifoliate stock into Texas in order to supply the large demand for trees for Citrus plantings. Whether it is indigenous to Japan is not known, but it is probably native of parts of eastern Asia. Since its introduction into Texas it has been disseminated by the shipment of diseased trees to other States and has further been introduced by shipments to these States direct from the Orient, so that it now occurs in parts of Florida, Alabama, Mississippi, Louisiana, and Texas.

Citrus canker had probably been present in the United States for five or six years before it was recognized as a new Citrus disease. Specimens were first collected in September, 1912, but it was not until July of the following year (1) that the Office of Nursery Inspection of Florida realized that these specimens did not represent an unusual manifestation of scab caused by *Cladosporium citri*. This mistake in diagnosis had also been made by inspectors in other Gulf States and by officers of State Experiment Stations and of the Federal Department of Agriculture. Japanese authorities had also mistaken this disease, since specimens received at the Florida Agricultural Experiment Station (2) from Japan had been identified as scab. The disease was brought to the writer's attention in February, 1914, and has been interruptedly studied by him since that time.[2]

A number of publications upon Citrus canker, all preliminary in nature, have appeared. These papers call attention to the presence of the disease in the several States, briefly describe its appearance, and recommend concerted cooperation in its eradication. The disease was first regarded as of fungoid origin, and the first claim that bacteria are the primary cause of the disease was made by Hasse (6). The present publi-

[1] Reference is made by number to "Literature cited," pp. 98–99.

[2] The writer severed his connection with the Alabama Agricultural Experiment Station on January 1, 1916. This study therefore is incomplete, time not having been afforded for verification of all portions of the study, and certain problems which have appeared in connection with the work have not been investigated. However, it was deemed advisable to record the results of the studies thus far conducted.

cation has for its purpose the recording of studies which are in part confirmatory of previous studies (2, 5, 6, 16, 19) and which further contribute to our knowledge of this disease.

ECONOMIC IMPORTANCE

The serious nature and unusual virulence of Citrus canker and the jeopardy in which it has placed the Citrus industry can best be realized when it is recalled that the Federal Horticultural Board, on January 1, 1915, placed a quarantine on the importation from all foreign countries of Citrus nursery stock, including buds, scions, and seeds, in order to prevent further introduction of the disease into the United States. It is difficult to obtain figures as to the number of nursery and orchard trees which have been destroyed in an effort to eradicate the disease from the Gulf coast. It is equally difficult to obtain accurate figures on the amount of money which has already been expended by the Federal Government, together with the State horticultural boards, liberally aided by various organizations and by private subscriptions, in an effort to stamp out Citrus canker. Suffice it to say that the actual cost in money for eradication and for trees destroyed has been enormous.

SYMPTOMS OF THE DISEASE

Citrus canker affects the leaves, twigs, larger branches, and fruits in a characteristic manner. Upon any of these parts the diseased areas are light brown in color and project more or less above the surrounding tissues. The cankerous areas consist of a corky mass of cells covered by a lacerated grayish membrane. It can be determined with certainty without a microscopic examination in case one has typical diseased material, and in case one has seen the disease in the various stages of development under field conditions. It is sometimes impossible to be certain whether meager specimens such as are sometimes sent in for identification are affected with canker or with some other leaf trouble. This is especially true in the case of canker on the Satsuma orange. If, however, one is permitted to make a field examination, and can thus learn of the origin of the trees, and can also observe adjacent trees, typical material may be found if Citrus canker is present.

OCCURRENCE ON THE LEAVES

The first evidence of canker on the leaves is the appearance of very small oily or watery dots on the lower leaf surface. They may appear on either surface, but are more commonly found on the lower leaf surface. They are of a darker green color than the surrounding leaf tissue and may at this stage be mistaken for oil glands (fig. 1, 2). The diseased areas are slightly convex, however, and within a few days will have extended through the leaf, appearing on the upper surface as

greenish yellow spots. By continued development the convex surface of the spots comes to be more and more elevated until the epidermis is broken by the increased tension and the subjacent tissues are thus exposed to desiccation. The exposed tissues then become corky, darkening with age. The ruptured epidermis is turned back irregularly and persists as a lacerated membrane. The margin of the diseased area maintains an oily appearance even after the spots have ceased to increase in size. Mature spots (Pl. XI, fig. 1) vary in size from very minute to a quarter of an inch in diameter and are typically circular in outline. They may occur singly; or when they are very numerous, fuse, thus forming large, irregular areas. Cankered areas are typically elevated on both leaf surfaces. In the case of canker on Satsumas (Pl. VIII, fig. 2, and Pl. IX, fig. 4), however, there is little or no elevation of the upper leaf surface. Neither is the oily margin so evident on this host, especially in case of old cankers, in which diseased tissues have become dark brown, simulating the appearance of melanose. The appearance of the disease on leaves of *Citrus trifoliata* as shown in Plate X, figure 1, is very similar to that on grapefruit. Stevens (2) reports that he has never found Citrus canker on trifoliate orange leaves. The uninvaded tissues surrounding the cankers are paler green than the normal tissue and gradually form a chlorotic or yellowish zone (Pl. VIII, fig. 1, and Pl. X, fig. 6), which may invade all the tissues not actually occupied by the cankers. At this stage considerable defoliation, especially in the case of grapefruit and trifoliate oranges, may occur. Cankers on the leaf petioles cause defoliation even though the leaves are otherwise uninvaded.

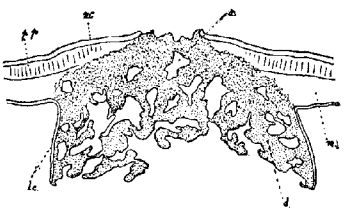

FIG. 1.—Diagrammatic representation of young open type of Citrus canker of half the diameter of the one shown in figure 2. *pp*, Palisade parenchyma; *ue*, upper epidermis; *le*, lower epidermis; *d*, diseased tissues; *a*, air space arising from tensions due to the enlargement of cells and disintegration of tissues.

OCCURRENCE ON THE TWIGS AND BRANCHES

Limb canker appears more commonly on very young twigs because of the absence of any considerable suberization, but larger branches are subject to infection. Growing cankers have been observed on limbs ½ to ¾ inch in diameter (Pl. VIII, fig. 3, 4). The disease has been found on branches of grapefruit, trifoliate oranges, lemons, Satsumas, and certain varieties of round oranges. Cankers on twigs are first apparent as small, circular, watery spots. They rapidly enlarge, become blister-like and the epidermis ruptures, exposing the cankerous tissue below. At this stage they project more or less prominently and are very similar

in appearance to the spots on the foliage. Isolated cankers remain circular in outline. When the spots originate close together, however, large irregular, variously cracked or fissured cankers are developed, which may involve an area several inches in length. The epidermis persists as a grayish broken membrane at the margin of these cankers (Pl. VIII, fig. 5). Twigs and larger branches may be completely girdled, resulting in the death of the distal parts. Affected trees exhibit a stunted growth, and numerous branches may be developed below the dying tips.

The disease is very severe upon stems of grapefruit and trifoliate oranges. On the latter host the thorns are abundantly cankered and the base of the thorns appears commonly to be the initial seat of infection.

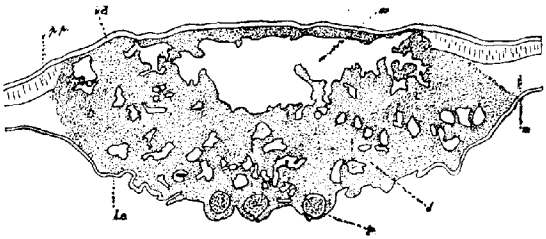

FIG. 2.—Diagrammatic representation of canker on old Citrus leaf: *pp*, Palisade parenchyma; *ue*, upper epidermis; *le*, lower epidermis; *p*, pycnidium of *Phoma socia*; *d*, diseased tissues; *a*, air space arising from tensions due to the enlargement of cells and disintegration of tissues.

Limb cankers on trifoliate oranges oftentimes are zonate with different shades of brown, especially if the outer membranes have not yet been ruptured.

OCCURRENCE ON THE FRUIT

The cankerous areas on the fruits are quite similar in appearance to the leaf cankers, differing mainly in the larger size of the former. They are scurfy elevations, for the most part circular in outline and surrounded by a zone of chlorotic rind tissues. The corky diseased tissues are quite superficial; and if the spots unite, large scaly areas are formed (Pl. X, fig. 2). In this case the fruits may crack open because of their increase in size owing to the growth of the fruits and may become prematurely yellow and drop. Fruits which are badly cankered and have burst open are, of course, subject to invasion by various organisms of decay. Even if they remain on the tree, they are rendered very unsightly and are unsalable.

OCCURRENCE ON THE BUDS

Nurserymen experience considerable losses from failure of Citrus buds to unite with the stock. In some cases when *Citrus trifoliata* seedlings affected with canker are used as stock, losses of over 50 per cent have

been sustained. The operation of budding either directly conveys the organisms into the wounded tissues or they are subsequently washed into them from cankers above the insertion of the bud before union has been effected.

ETIOLOGY OF THE DISEASE

The primary cause of Citrus canker is a bacterial parasite, *Pseudomonas citri* Hasse (6). Hasse isolated this organism from cankers on grapefruit and proved it to be pathogenic to grapefruit seedlings. This claim was established at a time when the disease was regarded as of fungus origin. Hasse further pointed out the fact that a number of fungi were isolated from old Citrus cankers. The writer had found a fungus, as had also Prof. H. E. Stevens, of the Florida Agricultural Experiment Station, belonging to the form genus Phoma, commonly associated with cankerous tissues. The writer's initial inoculations were made not with pure cultures of Phoma, as has subsequently been learned, but with cultures which had overrun the bacterial parasite. Successful infections reported in the previous publication (19) are thus accounted for. Consideration will be given in another part of the present report to the part which *Phoma* spp. and certain other fungi play in the production of Citrus canker.

PATHOGENICITY

Pseudomonas citri has repeatedly been isolated during the past season from cankers on grapefruit, trifoliate orange, lemon, and Satsuma oranges. The strains from these different hosts present the same cultural characters. Because of this, together with the added fact that no difficulty has been experienced in making cross inoculations, the strains are regarded as identical.

The plants used in making the inoculation experiments were grown in the greenhouse at Auburn, Ala. Typical cankers have been produced on McCarty and seedling grapefruits (Pl. IX, figs. 1, 2), pineapple oranges, Satsuma oranges, and seedling trifoliate oranges. Infections on all these species were as readily secured, whether the organism had been isolated from *Citrus trifoliata*, Satsuma, grapefruit, or lemon. Neither was there any evident difference in virulence of any of the strains. A suspension of the organism taken from pure cultures grown either on potato cylinders or in bouillon was used in making the inoculations. This suspension when applied with an atomizer resulted in a high percentage of successful inoculations. A greater number of successful inoculations were secured, as would be expected, when the plants were covered with bell jars to prevent the too rapid evaporation of the moisture. When the inoculum was introduced into the tissues of leaves, stems, or fruits through needle punctures, cankers developed in all cases. In some cases the suspension was applied to leaves with the fingers. They were dipped into the suspension

and the material was then applied by gently rubbing the leaves between the thumb and fingers. In a few cases it was arranged so that twigs bearing young leaves could be immersed for an hour or two in a bacterial suspension. Leaves inoculated in this manner are shown in Plate VIII, figure 2. It is to be noted that the infections are so numerous as to involve the greater part of the lower leaf surface.

The period of incubation appears to vary, depending on temperature, moisture, and age of the plant tissues. Very definite signs of the disease have been noted within 72 hours after inoculation. In other cases 10 days were required before the infections were evident to the eye. The longest periods were secured on Satsumas.

An organism of the same color as *Pseudomonas citri* and similar in appearance on certain media, but which does not exhibit the characteristic growth of *P. citri* on potato cylinders, has commonly been isolated from old cankers. This organism has not been found to be pathogenic on species of Citrus, however. There can be little doubt of the pathogenicity of the organism concerning which Hasse made her preliminary report (6). It is to be noted that her Plate X, figures *A*, *B*, represent natural infections and Plate X, figure *C*, artificially produced cankers. These, however, are regarded as identical in appearance. Artificially inoculated seedlings are represented also in Plate IX. As can readily be seen, the artificial cankers are much more prominently projecting than natural ones, are evidently greenish white in color, and there has been no discoloration of the leaf tissue surrounding the spot. The writer has never, under field conditions, seen specimens which resembled these artificial inoculations represented in Hasse's Plates IX and X, and he, furthermore, has examined fresh specimens in various stages of development sent from Florida, Alabama, Mississippi, Louisiana, and Texas. However, cankers similar in appearance to Hasse's artificial cankers have been produced in the greenhouse. Following her suggestion that the open, spongy type of canker is due to favorable conditions of moisture and temperature, seedling grapefruit which had been atomized with a suspension of *P. citri* were kept continuously covered with a bell jar. They were watered sufficiently often so that the air under the bell jar was maintained at a high relative humidity. Within 10 days the cankers shown in Plate IX, figure 3, had developed. These are regarded as similar in appearance to those previously produced by Hasse and represented in her Plates IX and X.

DESCRIPTION OF PSEUDOMONAS CITRI

The primary cause of Citrus canker is a yellow, 1-flagellate organism. Its motility can be observed when taken directly from young cankers and examined in a drop of water. In this case it will be found to occur singly or in pairs. On solid media it may form into chains of six or more elements. It is quite variable in shape and size. When taken

from young cankerous tissues it is usually a short rod with rounded ends which measures from 1.5 to 2.5 by 0.5 to 0.75μ. In old cultures the elements may be ellipsoidal. No endospores have been demonstrated; nor have involution forms been observed.

The organism stains readily with solutions of carbol fuchsin, analine gentian violet, and methylene blue. Only negative results have been secured with Gram's stain. When the organism has been grown on potato cylinders and is stained with anilin gentian violet, it has an apparent capsular portion (fig. 3). This capsular portion gives rise, no doubt, to the viscidity which characterizes its growth on steamed potatoes. The slime on old potato cultures can be drawn out an inch or two and does not dissolve readily in liquid cultures.

Young cultures of this organism on steamed potato cylinders have a very characteristic appearance. The growth is bright yellow, smooth, moist, glistening, and raised, with a narrow white zone along the margin of the bacterial growth. This white margin does not persist, since by its rapid growth the organism covers the entire surface of the medium. It acts very strongly on potato starch, as indicated by the entire absence of an iodin reaction on steamed potato cylinders 6 to 8 weeks old. The middle lamellæ in such old cultures have been dissolved, and the empty cells can readily be separated from one another.

FIG. 3.—*Pseudomonas citri*: *a*, Stained with carbol fuchsin; *b*, stained with Williams's flagellar stain (adapted from Hasse); *c*, stained with anilin gentian violet.

The organism has been grown on nutrient agar made by adding a water extract of corn meal, bean meal, green beans, cowpeas, potatoes, rice, orange juice, or orange leaves and stems, but the growth on none of these media is characteristic. No attempt was made to titrate any of these media to determine their acidity or alkalinity.

Colonies appear on the second day in poured plates of green-bean agar kept at room temperature. Within four or five days the surface colonies in poured plates will have become 2 or 3 mm. in diameter. The margin of the colonies is entire, and they are opaque yellow in color. They are appreciably raised and have a smooth, wet-shining surface. The character of the margin and of the surface is shown in Plate XI, figure 4. It will be noted that the reflection of the two windows in the room in which the exposure was made is shown in each of the colonies.

A filiform growth, following the line of the stroke and widening at the base of the slant, is formed in stroke cultures on green-bean agar. The growth does not penetrate the agar and does not give rise to the production of any stain or odor. In stab cultures on this medium a filiform but otherwise nontypical growth is produced, which when viewed from

above appears like the surface colonies in poured plates. Growth in stab cultures on various media is always or nearly always best at the surface of the media.

On nutrient-gelatin plates the colonies are circular in outline, slightly raised, entire margined, and yellowish. In gelatin stabs a filiform growth appears along the line of puncture, with the greatest growth at the surface of the medium, and with a rather slow liquefaction.

The organism is regarded as a facultative anaerobe. No gas is formed in fermentation tubes containing a 2 per cent solution of Witte's peptone. With this as a basal solution, five solutions were made by adding 1 per cent of one of the following carbon compounds: Saccharose, dextrose, lactose, maltose, and glycerin. All inoculated tubes developed a slight cloudiness, which extended into the closed end of the tube by the second day. More vigorous growth occurs in the open end of the tube, however, and after four or five days the cloudiness is very marked. Yellowish flocculent particles appear later in the open end, and a yellowish ring is formed at the surface. No gas formed in any of the solid media in which the above-mentioned carbon compounds were added to the nutrient agar.

In stab cultures on litmus-dextrose, litmus-lactose, litmus-saccharose, and litmus-glycerin agar no gas formation was apparent in 10-day old cultures. It is not known whether acidification will occur in old cultures on these media.

In sterile tubes of litmus milk there is a rather slow reduction of the litmus. After five days there is a slight increase in the blue color. The reddish whey is gradually formed on the surface, and the casein is precipitated.

There is no reduction of nitrates in Witte's peptone solution containing a trace of potassium nitrate. Phenoldisulphonic acid was used as a reagent 10 days after the date of inoculation, at which time both the check and the solution in which the organism was growing were colorimetrically alike.

Only negative tests for indol were secured in peptonized beef-bouillon cultures. A very conspicuous clouding occurs in this medium within 24 hours after inoculation. As these cultures get older they become somewhat flocculent, and a yellowish ring is formed at the surface of the media.

The thermal death point, as found in preliminary tests, was between 58° and 70° C. In order to determine more nearly the point, tests were made by exposing the organism taken from potato cylinder cultures, and transferred to tubes of bouillon. The tubes were then placed in a water bath for 10 minutes at some given temperature between these limits. The temperature of the bath was kept constant during the period of exposure. The tubes were subjected to room temperature for several

days to observe the development of cloudiness. In order to be certain, however, of the viability of the organism, loops of bouillon from these tubes were transferred to planted plates of nutrient agar, and the subsequent development noted. No growth occurred in the tubes exposed at temperatures above 65° C.

No attempts have been made to determine the exact degree of tolerance of this organism to acids. When transfers were made to dextrose-peptone agar +10, +20, and +40 Fuller's scale, it was found at the end of three days to have grown in the first two, but growth was completely inhibited in +40 acid. Hydrochloric and citric acids were employed in acidification.

The organism seems to exhibit a very considerable resistance to drying. In the desiccation experiments bacteria from vigorous pure cultures on potato plugs were smeared by means of a sterile platinum needle on clean miscroscopic slides in moist chambers. The moist chambers containing the microscopic slides were sterilized prior to transferring the bacterial smear to the slides. These preparations were made on June 1, and placed in a wall closet in the laboratory. On July 1, August 1, and September 1 several of the microscopic slides were removed from the moist chambers and placed in sterilized Petri dishes, using proper aseptic precautions in making the transfers. Tubes of melted nutrient agar which had been cooled almost to the point of solidification were poured upon these smeared slides. No growth occurred in the case of those tested on September 1, but those tested on July 1 and August 1 were still alive. From this it is believed that the organism can retain its viability for about two months.

The group number according to the descriptive chart of the Society of American Bacteriologists is 221.3332513.

LIFE HISTORY OF THE ORGANISM

Pseudomonas citri, so far as is known, passes its entire life cycle under natural conditions within the tissues of the host. New infections appear in spring shortly after the new growth has begun. In southern Alabama the first appearance of Citrus canker in the field was noted on May 11, in 1914, and on May 27, in 1915. Old diseased areas on the foliage together with the cankers on the twigs and larger limbs are undoubtedly the source of infection in the spring. New leaves formed near old twig cankers are especially liable to become diseased first. Infections are not confined to the new growth, however. Old diseased areas on leaves and branches may enlarge by the renewed growth of the organism which has remained dormant on the margin of the old cankers. New cankers may also develop on old foliage and twigs, especially near the old, actively growing cankers. Under favorable conditions new infections may appear at any time throughout the growing season of the host. In one instance

new infections are known to have appeared abundantly under field conditions during November, 1914. Old leaves on the ground may possibly harbor the organism and there it may remain viable for a long time. Unsuccessful attempts, however, have been made to recover the organism from leaves kept in the laboratory from September, 1914, to May, 1915; nor has recovery been possible in the case of twig cankers kept under laboratory conditions from March to October, 1915.

It is believed, moreover, that the organism survives the winter in fallen leaves and that these fallen leaves constitute a very important source of infection in the following spring, especially in the case of nursery trees which have been planted between diseased grove trees.

There is every reason to believe also that the organism can remain alive in soil. This is evidenced by numerous instances in which new sprouts have come up from the roots of diseased trees which had been burned. A large percentage of these sprouts are early found to be diseased. Furthermore, the leaves on the lowermost branches or those in actual contact with the soil are commonly the first to become diseased.

The fact that the stomata, or breathing pores, on species of Citrus occur only on the lower leaf surfaces and that infections developed only on the lower surface of the leaves in all of the inoculation experiments in which the plants had been sprayed with bacterial suspensions led to the inference that the canker organism must gain entrance to the leaves through the stomata. That such is the case was established by leaf sections which were fixed 72 hours after inoculation and which were subsequently properly infiltrated, cut, and stained (fig. 4). Lenticels very probably serve as portals of entrance for the organism into the stems. A film of moisture on the surface of the leaf, twig, or fruit enables the organism to move about and thus to gain entrance into the substomatal cavity. Under ordinary conditions inoculation will be successful only in the presence of moisture. Wounds or abrasions from any cause may afford an entrance to the bacteria. Inoculations not infrequently occur through wounds made by thorns. Inoculations on leaves made by thorn scratches are shown on Plate X figure 3. Thorns which come in contact with limbs near by may inflict wounds which have subsequently been observed to be the point of origin of limb cankers. Cankers have also been found at the point of contact of limbs which rub together through movement by the wind.

When once the bacteria have passed through the stomata into the substomatal cavities, they multiply rapidly and effect a passage between the host cells to the intercellular spaces which become filled with solid masses of bacteria. As the bacteria continue to multiply, the cells farther away from the substomatal chambers become involved seriatim. In this way an area circular in outline and extending entirely through the leaf comes to be invaded. Various stages of invasion of the leaf tissues have

been observed in serial paraffin sections. Within three to five days after inoculation the disease is evident in the form of oily or watery spots. Within another week with favorable weather conditions and on young leaves the epidermis will have ruptured on one or both surfaces and open cankers will have formed. At this stage, before the exposed cells have become desiccated, the greatest danger of spreading the infection exists. Young tender tissues seem to be more susceptible to infection at this time than mature tissues. The disease progresses more rapidly, too, in young tissues than in older parts.

FIG. 4.—Early stage of Citrus canker in cross section on a young leaf of seedling grapefruit. The leaf was inoculated by immersion in a suspension of *Pseudomonas citri* from pure culture. The material was collected 72 hours after inoculation. It was then killed in strong alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin. The organism entered the leaf through the stoma, multiplied in the substomatal chamber, and spread to adjacent intercellular spaces. Drawing made with a camera lucida. × 600.

RELATION BETWEEN PARASITE AND HOST

No study has been made other than the preliminary account of Hasse (6) of the effects of *Pseudomonas citri* on Citrus tissues. She states (p. 98) that—

> There is a rapid development of cells, and the tension resulting from the abnormal growth quickly ruptures the epidermis. The cells are found to be filled with short rod bacteria. All the cells exhibit more or less enlargement. In later stages in the development of the canker some of the cells disintegrate, and lesions are formed. The organism appears to act more vigorously on the cell contents than on the cell walls, and in due time the cell contents are exhausted. The cell walls which remain become suberized.

This problem was first attacked by making a histological study of the diseased tissues. For this purpose cankers in various stages of development on fruits and leaves were cut out so as to include some of the surrounding healthy tissue. Cankers which had developed under conditions of very high relative humidity (Pl. IX, fig. 3, and Pl. X, fig. 4) and which were consequently of the spongy type and white in color yielded especially interesting results. This white color is due to the presence of air between the cells and can be made to disappear if the cankers are immersed in water. These excised cankers were then killed in strong alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin. This stain renders the bacteria bright red, making it easily possible to determine their position within the tissues.

Contrary to Hasse's observation, the bacteria teem around and between the host cells, being present in especially large numbers in the intercellular spaces (fig. 5). When the organism occurs within the

cells, one is led to conclude, since they appear to be confined to such cells, that entrance was effected after some mechanical rupture of the host cells.

A microscopic examination of sections of young spongy cankers in which there has been no desiccation from contact with the air shows that the host cells are not killed at first. Instead, they are considerably hypertrophied and become lightly attached to each other, as shown in figures 6 and 7. In fact, if fresh cankers are cut off with a sharp razor and mounted on a slide in a drop of water, some of the host cells separate intact and of their own accord from the mass of cankerous tissue. Little if any hyperplasia is believed to occur. It is highly improbable that cell division would occur in cells in which such profound changes were taking place. It is evident from figures 6 and 7 that the enlargement of cells already present would account for the production of the cankerous tissues. The same is believed to be true in Plate X, figure 1, illustrating Hasse's observations. It is not clear, however, from her explanatory statement that "there is a rapid development of cells" whether hypertrophy or hyperplasia is meant. Death of cells in the later stages of development of canker is probably caused by drying (Pl. IX, fig. 5). The dried cankerous tissues gradually become suberized.

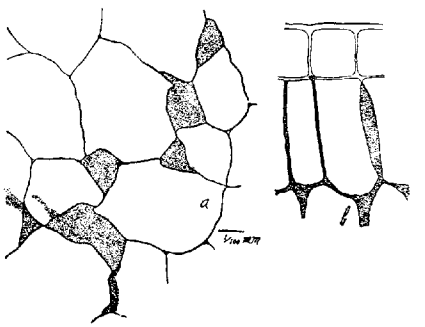

FIG. 5.—*Pseudomonas citri*: (*a*), In the mesophyll tissue and (*b*) in the palisade parenchyma. This material was fixed in strong alcohol, infiltrated with paraffin, sectioned, and stained with carbol fuchsin. Outlined with a camera lucida.

To explain the enlargement of the cells and their separation from each other, two hypotheses are advanced: First, the middle lamellæ are dissolved by an enzym, pectinase, secreted by the bacteria; second, osmotic pressure of the colloidal cell contents is modified so that the cells have a greater affinity for water. Evidence in support of both hypotheses has been secured which in part, at least, explains these interesting phenomena.

An attempt was made to demonstrate the secretion of pectinase by *Pseudomonas citri* by the following method: Six flasks of bouillon were inoculated with pure cultures of the organism. It was realized that the production of enzyms is largely dependent on the nature of the culture medium and that pectinase might be formed only within the host tissues. For this reason grapefruit leaves were placed in three of these flasks of bouillon prior to their sterilization and inoculation. After the organism

had grown in the flasks for four weeks, the bouillon was filtered through a Chamberland filter. This filtrate contained no living organisms, as demonstrated by transfers of platinum loopfuls to agar plates, with no growth on these plates after three days. When at the end of three days it was known that the filtrate was sterile, fresh grapefruit leaves were introduced into the filtrate. These leaves were sterilized, prior to their introduction, by immersion for half a minute in 1 to 1,000 bichlorid of mercury and by rinsing them subsequently in three changes of boiled tap water. Negative evidence of the presence of living organisms in the filtrate containing the grapefruit leaves was secured by agar plates made one week after the introduction of the leaves into the filtrate. An examination of the leaf tissues at the end of two weeks showed no evidence of dissolution of the middle lamellæ. This was true in the case of the filtrate obtained from both sets of the six original flasks.

In another experiment Irish potatoes were cut into slices and placed in moist chambers on moist filter paper. *Pseudomonas citri* was then

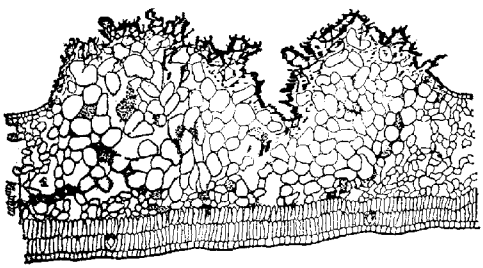

FIG. 6.—Drawing of a stained section of a natural canker on grapefruit.

transferred to these cut surfaces. Within a week hemispherical areas in which the cells were easily separable one from the other had been formed immediately beneath the colonies. That *P. citri* alone had caused this condition was shown by the reisolation in pure culture of this organism from the softened potato tissues. Because of this result, together with the fact, previously indicated, that the cells of cankerous tissues are so easily separable, and in spite of the negative evidence of enzym secretion in bouillon culture, it is believed that pectinase is secreted by the parasite.

The fact of the increased size of cells of cankerous tissue in itself supports the hypothesis that there has been an increased osmotic pressure within affected cells. Several facts contribute toward solving the question of how this increased pressure is brought about. In the first place the cell contents must manifestly be modified by the dissolution of the middle lamellæ, since there would be a tendency toward the establishment of equilibrium between the solution between the cells and the cell sap. Again, the growth of the organism between the cells with the consequent passage

of nutritive substances through the cell walls must exert an influence on the concentration of the cell sap. Then, too, the gelatinous material making up the bacterial cell walls certainly possesses considerable power of imbibition.

Further it has previously been pointed out that *Pseudomonas citri* exerts a strong diastatic activity when grown on potato cylinders. The production of this enzym has also been demonstrated by growth on starch agar prepared according to the method described by Crabill and Reed (4). Within a week a clear halo around the edge of the bacterial colony is formed on this substratum, thus making a striking ocular demonstration of dissolution of starch by the canker organism. If diastase, secreted by this organism, is readily diffusible through the cell walls, and it is reasonable to suppose that it is, it can convert the relatively insoluble starch into more soluble carbohydrates and thus increase the osmotic pressure of the cell sap.

It is not impossible that these several causes of increased osmotic pressure operating conjointly or separately may so profoundly modify the imbibitory properties of certain colloidal substances within the cells that their affinity for water is in consequence greatly increased.

No attempt has been made to determine the isotonic coefficient of the cell contents of the enlarged cells, but for the reasons just mentioned it is believed to be greater than that of normal cells.

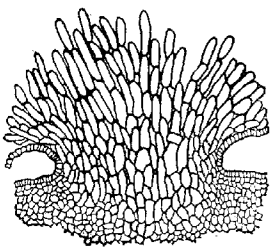

FIG. 7.—Cross section in outline of a spongy canker on the rind of a fruit of *Citrus decumana*, showing ruptured epidermis and hypertrophy of the rind tissues, the cells of which are loosely attached.

DISINTEGRATION OF THE TISSUES

An attempt has been made to gain certain information relative to the organisms involved in the disintegration of cankerous tissues, together with the nature of their activity on this tissue. It was previously pointed out that a species of Phoma is commonly associated with Citrus canker. Two other species of fungi belonging to the genera Gloeosporium and Fusarium are also sometimes present. Since certain bacteria and fungi are known to possess the power of hydrolyzing cellulose (13, 15), of which complex substance cell walls are largely constituted, an effort has been made to study the action of the organisms associated with canker upon pure cellulose. For this purpose cellulose agar was prepared according to the following method. Schweitzer's reagent was first made by adding ammonium chlorid and then an excess of sodium hydrate to a solution of copper sulphate. The blue precipitate thus formed was washed, pressed on a cloth filter, and dissolved in ammonium hydrate (sp. gr. 0.92). In this solvent 15 gm.

of sheet filter paper were dissolved, the solution was diluted about 10 times with water, and the cellulose was precipitated with a 15 per cent solution of hydrochloric acid. After considerable dilution the mixture was filtered, and the residue was washed repeatedly with water to remove all copper and chlorin. This residue was added to an agar medium consisting of agar, 10 gm.; monopotassium phosphate, 1 gm.; magnesium sulphate, 1 gm.; sodium chlorid, 1 gm.; ammonium sulphate, 1 gm.; calcium nitrate, 0.5 gm.; and the whole was made up to 1,000 c. c.

Poured plates of cellulose agar were made during May, inoculated with *Pseudomonas citri*, *Phoma* sp., *Gloeosporium* sp., and *Fusarium* sp., and incubated at room temperature. All grew poorly and none of the fungi fruited on this medium. There was no evidence of the production of cellulase except by *Phoma* sp. Within two weeks this organism had formed clear translucent halos as shown in Plate IX, figure 5, indicating that the cellulose had been hydrolyzed. Even though *Phoma* spp. strongly dissolve paper cellulose, they may not behave in this manner toward cell walls of *Citrus* spp., since other carbohydrates present would be more readily available than cellulose.

A further effort has been made to determine what other enzyms are secreted by these organisms and what part they might consequently play in the destruction of the tissues. Accordingly, Knop's mineral nutrient solution was prepared for use as a stock solution. This stock solution was then tubed and sterilized. To one set of these tubes of Knop's solution starch was added, to another saccharose, and to another maltose. They were then set aside and tested to determine whether they were sterile. It had previously been determined that sterilization subsequent to adding the carbohydrates resulted in a certain amount of conversion of these carbohydrates. When it was determined that they were sterile, four sets of four tubes each were taken of each of the nutrient solutions. Three tubes in each set of four were inoculated with pure cultures of one of the four organisms mentioned above and one tube in each set was left as a check. After 10 days the solutions were tested, with the following results: Fehling's solution showed a strong reduction in the starch solutions in which *Pseudomonas citri* and *Phoma* sp. had been grown, showing the production of diastase. There was no change in the checks nor in the solutions in which the other organisms were grown.

Inversion of saccharose, as evidenced on the reduction of Fehling's solution, had been accomplished in the solutions in which *Phoma* sp. and *Fusarium* sp. had been grown, indicating the presence of invertase. Positive tests for dextrose or glucose were secured with Barfoed's reagent and with Nylander's reagent in these inverted saccharose solutions. Negative results were secured with the other organisms and with the checks.

Phoma sp. alone seemed to have any action on maltose. Inversion into dextrose was shown by positive tests with Barfoed's reagent.

Negative tests for lipase production were secured in the case of each of the four organisms.

From the foregoing tests it is seen that *Phoma* sp. secretes cellulase, diastase, invertase, and maltase, and must therefore be regarded as very destructive to the carbohydrate material of diseased tissues. Cellulase very probably aids in the destruction of the cell walls; diastase converts the starch into maltose and dextrin and then further acts on the dextrin. When a few drops of iodin were added to a starch solution in which *Phoma* sp. had grown, blue and red colors developed, indicating amylo- and erythro-dextrin. Maltase probably further reduces the maltose to dextrose.

It has also been found that *Phoma* sp. affects the acidity of the medium upon which it is grown. This was determined by growth in pure culture of the fungus on leaves and fruits of *Citrus trifoliata*. This material was first macerated by passing it through a meat chopper. Thirty-gm. samples of ground leaves and of fruits were then placed in 250 c. c. Erlenmeyer flasks and were sterilized in an autoclave. After sterilization some were inoculated with *Phoma* sp. from pure cultures and others left as checks. A copious white growth occurred on those which had been inoculated. After a month 150 c. c. of distilled water were added to each of the flask cultures and to the checks. The flasks were then heated on a water bath for 30 minutes, the liquid filtered through asbestos, and 25 c. c. of the filtrate taken for titration, using *N/20* sodium hydroxid, with litmus paper as an indicator. The following is representative of the results obtained: 7.1 c. c. of *N/20* sodium hydroxid neutralized 25 c. c. of the filtrate from the leaves in the check flask and 9.8 c. c. that from the fruits in the check flask. The filtrate from the leaves upon which *Phoma* sp. had been growing was neutral to litmus, and that from the fruits required 1.3 c. c. of *N/20* sodium hydroxid to neutralize it. From this it is concluded that *Phoma* sp. is able to utilize the organic acids as a source of food, a condition contrary to that which Hawkins (7) found in a study of the chemical changes produced by the brown-rot fungus on peaches.

TAXONOMY OF THE FUNGUS

An effort has been made definitely to assign this species of Phoma to one of the numerous species of the form genera Phoma and Phyllosticta, which have previously been described as occurring on parts of *Citrus* spp. The pycnidia of the species under consideration are globose, ostiolate, 100 to 150μ in diameter (Pl. XI, fig. 5, 6) and wholly or partially embedded within the cankerous tissue. The pycnidial walls are thin, being thickest around the ostiolum, and are very similar in color to the corky brown host cells. The conidia are elliptical or oblong in outline,

hyalin, and 9 to 12 by 3 to 4μ. They germinate within 24 hours in water or in a variety of culture media. A white mycelial growth is produced on bean agar. Pycnidia are readily formed on agar (Pl. XI, fig. 3) modified by the addition of a water extract from corn meal, rice, cowpeas, orange stems, etc. (fig. 8).

The fungus, furthermore, was very probably introduced into the United States simultaneously with *Pseudomonas citri*. It is impossible to determine the position of this organism among previously described species, since it has been found to be morphologically not unlike several of them. Its relation to the production of Citrus canker is definitely established as a result of this study. Then, too, no particular difficulty would be experienced by other investigators in identifying it because of its association with Citrus canker. In view of these facts it seems well to describe it as a new species with the following brief technical diagnosis:[1]

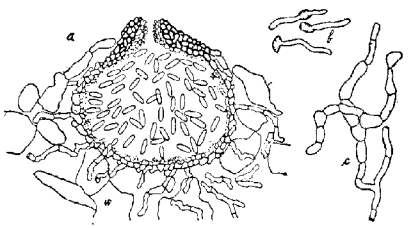

FIG. 8.—a, Cross section of a pycnidium of *Phoma socia* from a grapefruit leaf. This material was fixed in chromo-acetic acid, embedded in paraffin, sectioned, and stained in saffranin and gentian violet. Drawing outlined with the aid of a camera lucida. *b*, Germination of conidia of *Phoma socia* after 24 hours in water. *c*, Mycelium of this fungus in old cultures.

Phoma socia, n. sp.

Pycnidia irregularly distributed, globose, wholly or partially embedded, 100 to 150μ in diameter; walls thin, corky brown in color, thickened only around the ostiolum, which opens centrally; conidia continuous, elliptical or oblong, hyalin, 9 to 12 by 3 to 4μ.

Occurs in the cankers produced by *Pseudomonas citri* on living leaves and branches of *Citrus trifoliata*, *C. nobilis*, and *Fortunella* sp. and on living leaves, branches, and fruits of *C. decumana* and *C. aurantium*.

ACIDITY AND RESISTANCE TO CANKER

It is generally conceded by both nurserymen and growers and has been substantiated by the field observations of the writer that Satsuma oranges are not as susceptible to Citrus canker as grapefruit. This difference may be noted when both species are grown in locations where they are equally exposed to infection. The tolerance of bacteria to acidity has been found to be relatively low. Resistance to certain fungus diseases, as, for example, the resistance of hard wheat to rust, has been found (3)

[1] **Phoma socia,** sp. nov.

Peritheciis irregulariter distributis, globosis plus minusve immersis, 100 to 150μ diam.; contextu membranaceo, corticale-brunneo, cum cellulis circa ostiolum pseudoparenchymaticis, centro perforatis; sporulis continuis, ellipticis v. oblongis, hyalinis 9–12 × 3–4μ. Hab. in foliis ramisque, vivis *Citri trifoliatae*, *C. nobilis* et *Fortunellae* sp. et quoque in foliis, ramis fructibusque *C. decumanae* et *C. aurantii*. Socia adest *Pseudomonas citri* Hasse.

to be correlated with the acidity of the cell sap. Because of these several facts, an effort has been made to determine whether the difference in susceptibility between Satsuma oranges and grapefruit can be accounted for on the basis of difference in acidity. Leaves collected from plants growing in the greenhouse were used in these tests. The leaves were finely macerated by trituration; distilled water was then added to make a volume equaling 200 times the weight of the finely ground leaves; phenolphthalein was added as an indicator; and the acids present in the sample were titrated with *N*/10 sodium hydroxid. This method is open to criticism where absolutely accurate determinations are sought, but is regarded as satisfactory in indicating relative differences. Considerable variations in acidity of the same species were noted, dependent largely upon the cessation of photosynthetic activity at night. Greater acidity, as would be expected, occurred in samples collected early in the morning. Representative results of these tests, however, are shown in Table I.

TABLE I.—*Acidity of oranges and grapefruit*

Variety.	Wet weight of tissue.	Quantity of *N*/10 sodium hydroxid to neutralize 1 gm. wet weight of tissue.		Percentage of moisture in sample.	Percentage of total acidity based upon average water content.
		Actual.	Average.		
	Gm.	*C. c.*	*C. c.*		
Satsuma (old)	3.62	1.0359	1.0184	60.2	1.691
	3.06	.9967			
	4.14	1.0406			
	4.17	1.0431			
	2.92	.9760			
Satsuma (young)	2.60	.9423	.9735	67.8	1.465
	2.90	1.0172			
	2.55	.9609			
Grapefruit (old)	2.71	.8672	.8787	59.0	1.490
	2.53	.8695			
	1.66	.8434			
	2.11	.9005			
	1.95	.9128			
Grapefruit (young)	1.97	.8629	.8634	79.9	1.080
	2.03	.8620			
	1.56	.8653			

The leaves of Satsuma oranges are consistently higher in acid content than those of grapefruit, since the former require 1.0184 c. c. of *N*/10 sodium hydroxid to neutralize 1 gm. of wet weight of leaf tissue, young Satsuma leaves, 0.9735 c. c., old grapefruit leaves, 0.8787 c. c., and young grapefruit leaves, 0.8634 c. c. When the acidity of the cell sap is computed on the basis of the total moisture content of the leaves, it is found to be 1.691 per cent for old Satsuma leaves, 1.465 per cent in those of young Satsumas, 1.490 per cent in those of old grapefruit, and 1.080

per cent in those of young grapefruit. It will be recalled that bacterial growth occurs on artificial media rendered acid by hydrochloric or citric acid when a sufficient amount of acid has been used to make the acidity of the media 2 per cent. The acidity of the leaf tissue is therefore not sufficient to inhibit the growth of the canker organism and is not regarded as sufficient to account for the difference in susceptibility. No determinations have been made of the kinds and relative amounts of the several organic acids in the tissues of the two species. Until this is known there still remains the possibility of a correlation between susceptibility to canker and acidity.

CHEMICAL CHANGES IN CITRUS LEAVES BROUGHT ABOUT BY CITRUS CANKER

Little attention has been given by the biochemist to the chemical transformations occurring in diseased plant tissues. Such studies would no doubt throw a flood of light upon the intimate relationship of parasite and host and would materially contribute to our knowledge of the nature of parasitism. The literature dealing with the chemical changes induced by plant pathogens is more or less fragmentary, mainly because of the inexact state of our knowledge regarding the separation and quantitative estimation of the various compounds occurring in plant tissues. An historical résumé of this literature has therefore been purposely omitted. However, among the recent excellent papers along this line may be mentioned the work of Hawkins (7) upon the changes in peaches induced by the brown-rot organism, *Sclerotinia cinerea*. He found in brown-rotted tissues an increase in acid content, a decrease in certain alcohol-soluble substances, a decrease in the total sugar content, and practically a disappearance of the cane sugar. It was with the view of determining something of the changes produced by Citrus canker that this portion of the investigation was undertaken.

Diseased and healthy leaves were taken from grapefruit trees affected with Citrus canker. Circles of diseased tissue and tissue from healthy leaves were excised with a cork borer. These leaf circles were then triturated in a mortar until the material was finely divided, their wet weight determined, 27.25 gm. in each case, and preserved in such volume of 95 per cent alcohol that the alcohol concentration of the mixture was 85 per cent. This concentration could not be accurately made until it had been determined that the moisture content of normal leaves was 61.69 per cent and that of diseased leaves 61.57 per cent. The material was then set aside for two weeks and was shaken occasionally to permit the gradual extraction of the cold alcohol-soluble portions. The method followed subsequently was based upon those devised by Koch (9, 10, 11, 12) for use in the quantitative chemical analysis of animal tissues.

This method consists essentially in the separation of the material into three fractions. Fractions 1 and 2 consist of the soluble portion extracted by the action of alcohol, ether, and water, and fraction 3 consists of the insoluble residue. Fractions 1 and 2 are separated by lipoid precipitation. The former fraction contains precipitated lipoids, while the latter contains all nonlipoid materials, soluble in alcohol, ether, and water. Instead of the modified Wiley extraction apparatus employed by Koch and his pupils, a rubber analysis extraction apparatus (8) has been employed. Extractions in this apparatus, like those with the modified Wiley apparatus, are carried out at the boiling point of the solvent.

In making the first alcohol extraction the preserved material was transferred to Schleicher and Schüll extraction thimbles, previously fitted into the siphon cups of the extraction apparatus. The preserving liquid was then filtered through these thimbles. Perforated porcelain plates or filter paper cut to fit were then used as covers over the material in the thimbles.

Extraction for 12 hours with redistilled 95 per cent alcohol followed. The alcohol was changed two or three times during this extraction in order to prevent possible decomposition of extracted materials in the boiling alcohol. The tissue was pressed to remove the excess alcohol, and an ether extraction was made. This extraction was continued for 12 hours for the purpose of facilitating the subsequent powdering of the tissues. The material was then removed from the extraction thimbles to a mortar and was ground to a powder. This powder was placed in a stoppered flask with a volume of distilled water equaling twice the fresh weight of the material and was boiled on a steam bath for two hours. Warm absolute alcohol was added in a sufficient quantity to bring the alcohol content of the whole up to 90 per cent. The mixture was warmed on the bath, with repeated shaking, and set aside until the following day. It was then filtered through the original extraction thimbles and extracted for 12 hours with 95 per cent alcohol. At the close of this extraction the residue in the cups (fraction 3) was transferred to previously weighed porcelain crucibles and dried to constant weight in an oven at 100° C. By this procedure the alcohol, ether, and water-soluble portions (fractions 1 and 2) were separated from the insoluble portion (fraction 3).

In further preparing the soluble portions of the material for analyses they were combined only after the ether-soluble portion had been heated on a water bath until the odor of ether could no longer be detected. A little alcohol was added from time to time to take up the materials left behind by the loss of ether by evaporation. In pouring the solutions together a precipitate appeared which was rendered soluble by the addition of sufficient hot water to bring the alcohol concentration down to 70 per cent. The solution was then made up to 2,000 c. c., 200 c. c. of which were taken for the estimation of solids. The remainder was

evaporated at 75° C. to a sirupy consistency or until all the alcohol had evaporated and the sirupy mass was emulsified with warm water. This emulsion was placed in a stoppered volumetric flask, shaken with 20 c. c. of chloroform, 10 c. c. of hydrochloric acid were slowly added, and then it was made up to a given volume by the addition of water. The flask was then placed for 48 hours in running water under the hydrant to facilitate the precipitation of the lipoids in the chloroform. Filtration followed, the filtrate constituting fraction 2, and the lipoid precipitate on the filter paper fraction 1. The precipitate was then taken up with a large volume of hot, 95 per cent alcohol, and kept on a water bath at 75 C., until all of the chloroform was driven off. The volume was then increased to a convenient amount, and aliquot parts taken for analyses.

The analysis of fraction 3 included (*a*) total phosphorus, (*b*) total nitrogen, (*c*) cellulose, (*d*) carbohydrate after hydrolysis, (*e*) ash, (*f*) total solids; the analysis of fraction 2 included (*a*) dry weight and ash made upon an aliquot part, (*b*) total sugars before and after hydrolysis, (*c*) total nitrogen, (*d*) phosphorus, (*e*) solids; while the analysis of fraction 1 included only (*a*) total solids, (*b*) phosphorus, (*c*) nitrogen, since the total weight of the lipoidal material from the two samples differed by 1 mgm. only and since the amounts were too small to admit of accurate separation.

The determinations of phosphorus were made upon aliquot parts by the Pemberton-Neuman method described by Mathews (14, p. 893–895).

The total nitrogen was determined upon all fractions by the employment of the Gunning-Arnold modification of the Kjeldahl method. No determinations were made of fatty acids.

In fractions 2 and 3 the carbohydrate determination included reducing sugars, total sugars, and cellulose. Prior to the determination of reducing sugars, the solution was freed from organic acids, tannins, and other substances capable of affecting reduction by Fehling's solution. This was accomplished by treatment with lead subacetate in excess, after which the solution was diluted, filtered, and saturated sodium sulphate was added to precipitate the excess of lead. The clear filtrate was then diluted, and an aliquot part taken for the determination of reducing sugar by the Bertrand volumetric method. The reducing sugar was calculated as dextrose by the Munson and Walker tables.[1] Another aliquot part of the solution, a part of which had been used for the determination of reducing sugars, was used upon which to determine the total sugars. This was hydrolyzed by the addition of concentrated hydrochloric acid, following which the solution was kept on a water bath at 69° to 70° C. for 10 minutes. It was then cooled, neutralized with 40 per cent sodium hydroxid, and the sugar determined as invert sugar by the volumetric permanganate method.

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 241–251. 1908.

Cellulose determinations in fraction 3 were made in duplicate with accordant results by employing Schweitzer's reagent in one case and a solution of zinc chlorid in hydrochloric acid in the other.

Polysaccharids in fraction 3 were estimated as dextrose after 2.5 hours' hydrolysis in a reflux condenser using 2.5 per cent hydrochloric acid.

In Table II are given the fresh weights of normal and cankerous Citrus tissue, moisture content, dry weight, and alcohol-ether soluble and insoluble portions.

TABLE II.—*Analysis of normal and cankerous tissue of grapefruit leaves*

Item.	Normal tissue.	Cankerous tissue.
	Gm.	*Gm.*
Fresh weight	27.250	27.250
Moisture	16.799	16.796
Dry weight	10.451	10.454
Total alcohol-ether:		
Soluble	4.270	4.300
Insoluble	6.181	6.154

In this composite table it is strikingly significant that only slight differences are apparent. The moisture content of normal tissue is slightly greater than that of cankerous tissue, and there is, of course, a corresponding decrease in dry weight. The greater amount of alcohol-ether soluble material occurs in cankerous tissues with a lesser amount of alcohol-ether insoluble substance. The differences represented herein would have little or no value in themselves if it were not that they were obtained by the use of a refined method of analysis primarily intended to permit the discovery of changes not indicated by ordinary methods. Studies of the intricate relation of parasite and host have proceeded far enough to indicate that large changes in composition of the host are not to be expected, but rather that transformations have been produced which, though minute in amount, profoundly affect the metabolism of both parasite and host. Table III gives in detail the results of the several steps in this analytical procedure.

TABLE III.—*Analyses (in grams) of normal and cankerous grapefruit leaves*

Item.	Fraction 1.		Fraction 2.		Fraction 3.		Totals.	
	Normal.	Diseased.	Normal.	Diseased.	Normal.	Diseased.	Normal.	Diseased.
Dry weight	0.781	0.780	3.479	3.520	6.181	6.154	10.441	10.454
Nitrogen	.0196	.686	.1190	.1120	.105	.0818	.2436	.2624
Phosphorus	.0123	.0114	.021	.0225	.0101	.0131	.0434	.0470
Reducing sugars			2.008	.806			2.008	.806
Sugar after acid hydrolysis			.387	.284	.573	.370	.960	.654
Polysaccharids (soluble)					.2087	.1273	.2087	.1273
Cellulose					.859	.843	.859	.843
Ash					.041	.041	.041	.041

It should be stated with reference to the data presented in Table III that the figures given are the weights in grams of the several constituents as determined by employing 27.25 gm., fresh weight, of healthy and of cankerous tissue. Since the two samples differed by only 3 mgm. in dry weight and since the figures, to be directly comparable, should be based on dry weights in each case, a correction of 0.24 per cent should be applied to the analyses of diseased tissue. As this is insignificant, the data are regarded as referable, and the corrections have not been applied.

Because of the presence of certain enzyms, of which mention has been made earlier in this paper, it is to be expected that the changes of greatest magnitude would occur in the carbohydrates. That such is the case is obvious when one notes in the totals given in Table III a reduction of all classes of carbohydrate in cankerous tissue. Thus, in equal quantities of fresh material the amounts of reducing sugar are found to be as 5 to 2, the total sugars as 3 to 2, and the polysaccharids as 5 to 3 when normal and diseased tissues are compared. Because of the ease with which they are available to the invading organisms, the reducing sugars are probably the most strongly attacked. After acid hydrolysis the normal tissue shows more reducing sugar than the diseased, both in the alcohol-ether soluble and alcohol-ether insoluble fractions. This means that there is also a less amount of the higher soluble carbohydrates, disaccharids, in diseased tissues and that they too are more easily available than the polysaccharids. The ratio of disaccharids in normal and cankerous tissue in the alcohol-ether soluble and alcohol-ether insoluble portions is as 3 to 2 and 5 to 3, respectively.

There is also a slight but significant decrease in the amount of cellulose found in diseased tissues. Although the difference in total cellulose in the normal and diseased tissues is slight, the results given are representative of a considerable number of determinations in which two standard methods were employed and in which the lesser amount of cellulose was invariably found in the diseased tissue. Experimental error has thus been eliminated and the results indicate a slight but unmistakable destruction of cellulose by the invading organisms.

The polysaccharids were determined in fraction 3 after 2.5 hours acid hydrolysis. They were found to be present in normal tissues and diseased tissues in the same proportion, 5 to 3, as were the disaccharids. There has therefore been a corresponding reduction and utilization of both di- and poly-saccharids by the invading organisms.

In the alcohol-ether insoluble fraction the amounts of nitrogen found for normal and diseased tissue were 0.105 and 0.0818 gm., respectively. If the conventional factor for these figures, 6.25, is employed, 0.654 and 0.511 gm. are obtained as the protein content of normal and diseased tissues, respectively. The protein content of diseased tissue has therefore been reduced 78.16 per cent. One should therefore expect to find a

very material increase in the nitrogen of the alcohol-ether soluble portion of the diseased tissue. This expectation is realized, since the nitrogen figures for the soluble portions are 0.1386 gm. for normal and 0.7806 gm. for diseased tissue. This represents an increase in the diseased tissue of 37.52 per cent over the healthy tissue. This increase in the soluble portion indicates a decomposition of the complex nitrogenous compounds resulting in the formation of peptones and amino acids soluble in alcohol and ether. This difference in nitrogen content of the alcohol-ether soluble portion takes an added significance when the nitrogen content of fraction 1 and that of fraction 2 are examined separately. It will be recalled that the nitrogen of fraction 2 represents those portions of the nitrogenous constituents extracted by alcohol and ether which are readily soluble in water after the combined extract has been evaporated to a paste. They are, therefore, amino acids and polypeptids. It will further be recalled that fraction 1 is obtained from the watery solution of the alcohol-ether soluble extract by chloroform precipitation and is therefore lipoid nitrogen. The slight decrease in nitrogen in fraction 2, when normal and diseased tissue are compared, is accompanied by an enormous increase, amounting to 250 per cent in the lipoid nitrogen of fraction 1.

These differences in nitrogen content of the several fractions lend themselves to two possible explanations. The first and most obvious interpretation of the results is that the changes produced by the invading organisms in the proteins of the host result in the formation not of amino acids and other end products of protein decomposition but in the production of complex intermediate substances. The other explanation is based upon the fact that the bacteria themselves derive the nitrogen necessary for the building of their own proteins as well as for the formation of their cell walls from the proteins of the host. Concurrently with the reduction of the protein of the host to simpler forms a series of metabolic processes is occurring within the invading organism which involves the synthesis of these simple nitrogenous compounds to more complex ones. The changes in nitrogen content of the several fractions of the diseased tissue are therefore the result of both analytic and synthetic processes. At present it is impossible to employ any methods, as none have been devised, which will indicate what the end products of decomposition of the host proteins by the invading organism are, since the formation of these products is accompanied by their concomitant utilization in the manufacture of new compounds peculiar to the body of the parasite.

The total phosphorus in the diseased tissues is greater in amount in fractions 2 and 3 than in the normal tissues. Were the changes in the diseased tissue purely katabolic, it would be expected that there would be a material increase in water-soluble phosphorus derived from the

decomposition of neucleoproteins. On the contrary, the phosphorus of fraction 3 shows an increase of 30 per cent, that of fraction 2 an increase of 20 per cent, and that of fraction 1 a decrease of about 7 per cent. The increase in water-soluble phosphorus in fraction 2 indicates that decomposition processes are taking place, but the concomitant increase in phosphorus content in fraction 3 shows that such decomposition is accompanied by actual synthetic processes involving the use of phosphorus.

No difference appears between the two tissues in amounts of ash as shown in fraction 3. The ashing of fractions 1 and 2 gave unsatisfactory results and for this reason the figures are withheld.

It is evident from the foregoing statement of results that the significant changes brought about in diseased tissues concern carbohydrate and nitrogenous constituents. The concurrent disappearance of mono-, di-, and poly-saccharids from diseased tissues indicates that all the sucroclastic enzyms previously shown to be formed by the organisms in pure cultures are active in the host tissues and that the reducing sugars formed are utilized by the organisms as sources of energy. The results with nitrogen indicate that there is not an accumulation of the products of protein decomposition but that the destructive transformation of protein is accompanied *pari passu* by a utilization of the decomposition products in the anabolic processes of the organisms.

AGENCIES CONCERNED IN DISSEMINATION OF CITRUS CANKER

Definite experimental data are wanting on the agencies by which Citrus canker is spread. If we judge, however, from field observations and from a knowledge of other bacterial plant diseases, it is evident that rain and dew are important factors in carrying the disease to unaffected leaves, twigs, and fruits of trees in which the diesase is already present. Man himself is a very important agent in effecting the distribution of canker from diseased trees to healthy trees near by. When in the cultural operations of budding, cultivation, picking, etc., he comes in contact with diseased trees and soon afterwards touches healthy ones, infection may result. The chances of infection are greatly increased if he comes in contact with newly formed cankers on the diseased trees, and if a film of moisture is present on the adjacent healthy trees which he may touch. The most plausible explanation of the introduction of Citrus canker into two groves which have come under the writer's observation is through the agency of man. The owners had visited groves in which canker occurred in order to acquaint themselves with the appearance of the disease. On returning home they examined certain of the trees in their own groves and these trees soon afterward developed canker lesions. Stirling (2) reports the transmission of the disease through handling diseased leaves prior to touching healthy ones. It is highly probable that

certain birds and insects also effect this contact of diseased with healthy parts and are therefore to be regarded as agents in dissemination of Citrus canker.

CONTROL OF THE DISEASE

During the summer of 1914 those who had been attempting to solve the problem of controlling Citrus canker realized that it was an exceedingly difficult undertaking. Efforts were directed along three lines: Exclusion, protection, and eradication.

EXCLUSION.—Those interested in the welfare of the Citrus industry in Florida were the first to realize the serious nature of Citrus canker and that it had been introduced into the State from other States and from foreign countries. For these reasons a quarantine was imposed during the spring of 1914 to prevent the further introduction into Florida of Citrus trees and buds and thus of Citrus canker. Other of the Gulf States later in the season realized the jeopardy in which their Citrus growers' interests were placed and issued similar regulatory measures on the importation of shipments of Citrus stock. On January 1, 1915, a Federal quarantine was imposed to exclude the further importation of this disease into the United States. The agitation throughout the entire Citrus growing section of the Gulf coast attendant on the adoption of these regulations looking toward control by exclusion have so familiarized the growers with Citrus canker that it is unnecessary to advise the exercise of care in ordering trees to be used in setting out a Citrus grove. It is realized that in no case is it safe to purchase trees from nurseries in which this disease occurs.

PROTECTION.—Since certain fungicides have been successfully used in the control of various Citrus diseases a number of experiments were undertaken during the spring of 1914 to determine the effectiveness of these mixtures in the control of Citrus canker. A grove of badly diseased grapefruit was used upon which to make applications of Bordeaux mixture, ammoniacal copper carbonate, and soluble sulphur. Details of these experiments are withheld, since it was realized early in the summer that the application of these fungicides was without appreciable effect in the control of canker.

Again in the spring of 1915 another grove of grapefruit was selected in which to test the effectiveness of several fungicides in protecting the trees from infection by the canker organism. All visible signs of canker were carefully removed from the trees prior to the application of the mixtures. Bordeaux mixture, Bordeaux mixture and bichlorid of mercury (12 tablets in 3 gallons), Bordeaux mixture and formaldehyde (1 : 100), and a Bordeaux and lead arsenate mixture were employed. Applications were made on March 26, April 29, and May 14, and no new infections had developed on any of the sprayed or unsprayed trees by the last-named date. On May 27, however, new infections were apparent

and were equally numerous on sprayed and check trees. A number of growers have used various germicidal mixtures in attempts to find a preparation which could be successfully employed against Citrus canker. In no case have these efforts met with a sufficient degree of success so that their use in canker control can be recommended. When formaldehyde is used in sufficient strength to cause the death of the leaf tissues in a considerable area surrounding the cankers no viable organisms can be found in the cankerous tissues in many cases. They are still viable, however, in others, and it has also been found to be impossible to cause formaldehyde to penetrate sufficiently deep into old suberized limb cankers to kill the canker organisms. In the light of these tests and in the light of the ineffectiveness of sprays in the control of other plant diseases of bacterial origin, it is believed that there is little to be hoped for in the use of germicides for protection against Citrus canker.

ERADICATION.—The history of the work of eradication of Citrus canker, little of which has been published outside of the daily press, would in itself be voluminous, and it is not the present purpose to include it in this account. The early efforts toward the eradication of Citrus canker were confined to the removal of diseased parts in case the trees were only slightly diseased. When the trees were seriously affected, however, they were severely pruned, even though this necessitated the removal of nearly all of the branches. Pruned trees were then thoroughly sprayed with Bordeaux mixture. It was recommended that all the diseased parts which had been removed should be burned.

After a few months' trial it was seen that by this procedure the treated trees were still diseased. Further than this, adjacent trees had become diseased, although they were apparently healthy at the time efforts had been made to remove cankered leaves and branches from the trees near by.

Even when the work of removal had been done by skilled hands and when the trees had received several applications of some fungicide to protect the new growth they were still found to become cankered.

As a result of this it was decided during the summer of 1914 that only the complete destruction of the diseased trees by burning would be effective. As a result of this decision the eradication campaign was organized and a concerted, heroic effort is being put forth to stamp out Citrus canker from the Gulf States. The intelligent observance of the strictest sanitary precautions with reference to trees adjacent to those which are destroyed is necessary.

SUMMARY

A serious disease, commonly known as Citrus canker, which affects species of Citrus and Fortunella, has within the past few years been introduced into Alabama and other of the Gulf States. It attacks fruits, leaves, twigs, and larger branches, producing characteristic cankerous

lesions. The primary cause of the disease is *Pseudomonas citri*, first isolated by Hasse from grapefruit and found to be pathogenic on grapefruit seedlings. This has been confirmed, and in addition an organism presenting the same cultural and physiological characters has been isolated from trifoliate and Satsuma oranges and lemons. No difficulty has been experienced in cross-inoculating the organism on McCarty and seedling grapefruit, Pineapple oranges, Satsuma oranges, and seedling trifoliate oranges. It grows readily on a variety of artificial media, and according to the studies made its group number is 221.3332513.

Infection occurs through natural openings and through wounds. The rapid spread of the disease is favored by the simultaneous occurrence of newly exposed cankerous cells and the presence of a film of moisture, especially on young parts of the plant. The bacteria occur for the most part between the cells of the host and cause them to become considerably hypertrophied. Little, if any, hyperplasia is believed to occur. This enlargement of the cells is caused by the dissolution of the middle lamellæ through enzym activity and by a modification of the host protoplast so that its osmotic pressure is increased. This increased pressure results from the presence of the parasite between the cells and from the passage of materials through the walls of the host, occasioned by the growth of the organism.

Besides *Pseudomonas citri*, fungi belonging to the genera Phoma, Fusarium, and Gloeosporium have been isolated from Citrus cankers. Of the fungi *Phoma* sp. alone was found to be notably active in the disintegration of the tissues. It is able by virtue of the secretion of specific enzyms to utilize the carbohydrates, cellulose, starch, maltose, and saccharose and causes also a decrease in acidity of invaded tissues. It is regarded as heretofore undescribed and is herein given the name "*Phoma socia*, n. sp."

The difference in susceptibility to Citrus canker of Satsuma oranges and grapefruit can not be accounted for on the basis of differences in total organic acids in the two hosts.

Comparative analyses of grapefruit leaves affected with Citrus canker and of healthy leaves shows that there has been in diseased leaves a decrease in all of the soluble and insoluble carbohydrates due to their utilization by means of sucroclastic enzyms secreted by the canker organisms. Apparently a decomposition of the host proteins occurs concurrently with their synthesis in the metabolism of the parasite proteins, and there results a slight increase in total nitrogen in diseased tissues. The slight increase in phosphorus in diseased tissues is accounted for in the same manner as that in nitrogen, since they appear to be correlated. No differences in ash were found in fraction 3, and the dry weight of diseased tissues was slightly greater than that of normal.

Rain and dew are important agencies in the dissemination of Citrus canker. Any other agencies, of which man is probably the most im-

portant, which effect a contact of diseased parts with healthy parts, are to be recognized as carriers.

In efforts to control the disease quarantine measures have been passed, thus preventing its further introduction from foreign localities and from any one of the Gulf States to any other of them. The use of spray mixtures indicates that they are not to be regarded as remedial measures of appreciable value in canker control; nor will their use protect healthy trees adjacent to diseased ones from infection.

Successful eradication seems possible, but only when the work of destruction of diseased trees is thoroughly done, with the observance of proper sanitary precautions.

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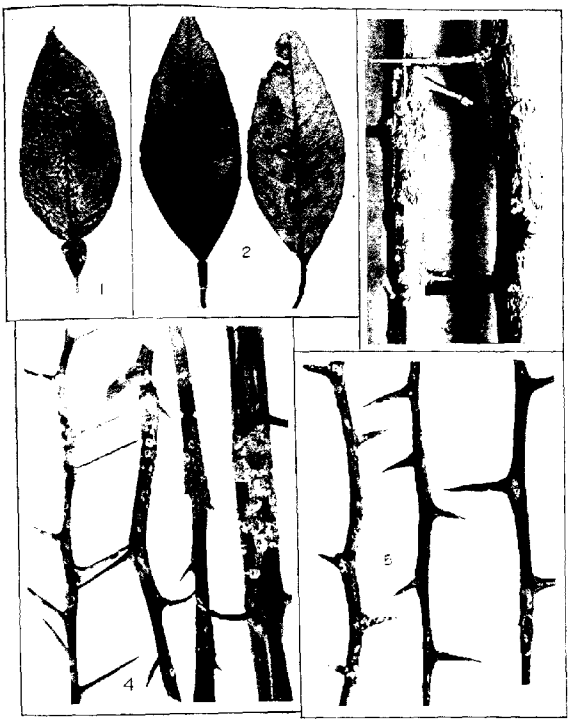
PLATE VIII

Fig. 1.—Grapefruit leaf showing young Citrus cankers.
Fig. 2.—Old Citrus canker on Satsuma leaves.
Fig. 3, 4.—Seedling grapefruit branches affected with Citrus canker.
Fig. 5.—Severe canker infection of branches of *Citrus trifoliata*.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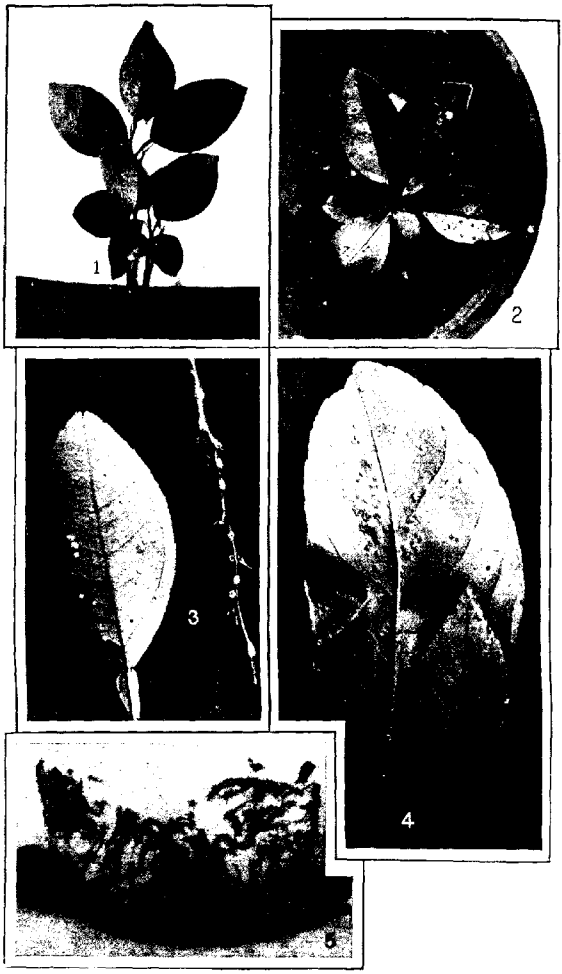
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PLATE IX

Fig. 1.—View of lower side of leaves of seedling grapefruit artificially inoculated with *Pseudomonas citri*.

Fig. 2.—Top view of plant shown in figure 1.

Fig. 3.—Spongy white cankers on leaf and twig of seedling grapefruit produced by artificial inoculation. The plants were continuously kept under a bell jar in a humid atmosphere.

Fig. 4.—Citrus canker on Satsuma leaves resulting from artificial inoculation with *Pseudomonas citri*.

Fig. 5.—Photomicrograph of section of young, open canker on grapefruit.

PLATE X

Fig. 1.—Natural Citrus canker infection on leaves of *Citrus trifoliata*.

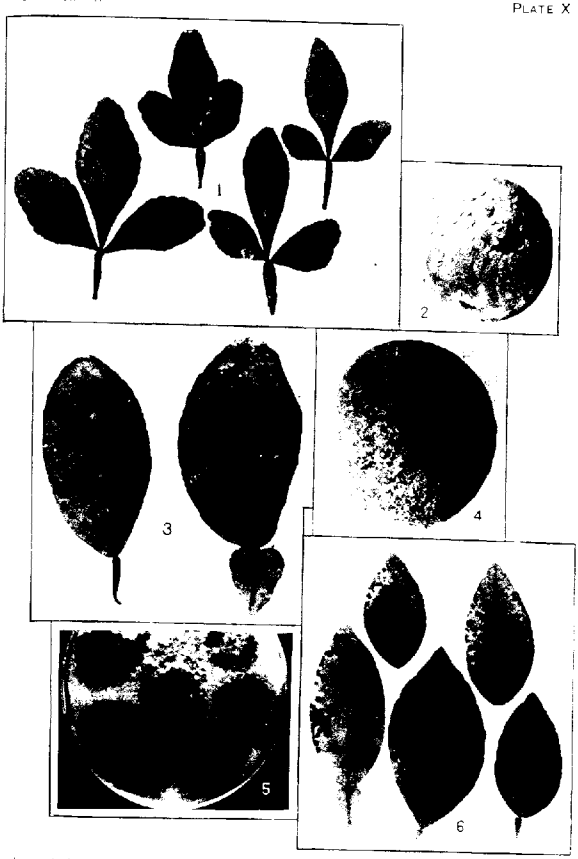
Fig. 2.—Mature cankers on fruit of *Citrus decumana* (courtesy of Dr. E. W. Berger).

Fig. 3.—Canker on seedling grapefruit leaves, entrance having been effected through abrasions made by thorns.

Fig. 4.—Young spongy cankers on fruit of *Citrus decumana*.

Fig. 5.—*Phoma socia* on cellulose agar showing dissolution of cellulose.

Fig. 6.—Mature cankerous areas on leaves of Duncan grapefruit.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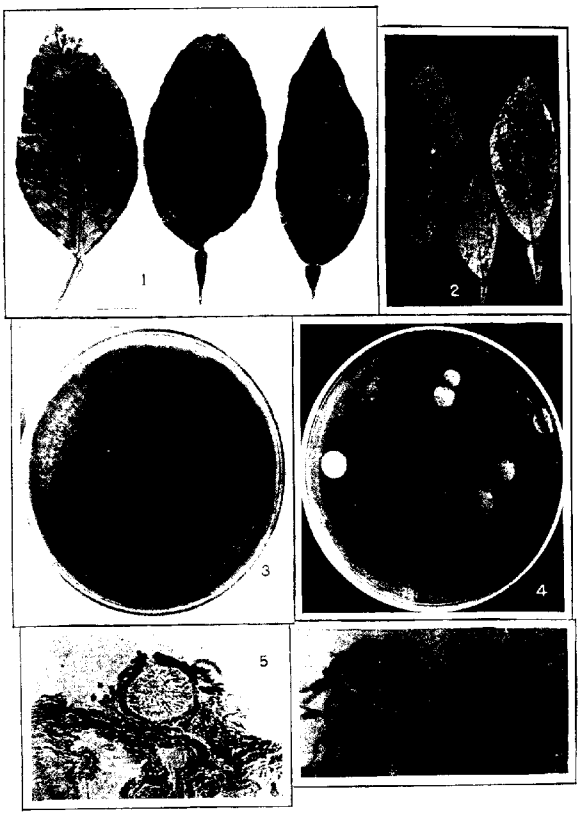
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PLATE XI

Fig. 1.—Cankers on old grapefruit leaves which have enlarged during the second growing season.

Fig. 2.—Citrus canker resulting from immersion of leaves in a bacterial suspension. Lesions involving a large part of the lower leaf surface are thus formed.

Fig. 3.—Culture of *Phoma socia* showing pycnidial formation in concentric rings.

Fig. 4.—Dilution poured plate of *Pseudomonas citri* on green-bean agar. The spots on the colonies are the reflection of the windows of the room in which the exposure was made. Colonies 14 days old, the last 5 of which days the plates were kept in an ice chest at a temperature of about 55°.

Fig. 5.—Photomicrograph of pycnidium of *Phoma socia* taken in reflected sunlight.

Fig. 6.—Photomicrograph of pycnidia of *Phoma socia* taken in diffuse light.

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